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(57) Abstract

Peptides which exhibit antimicrobial activity comparable to certain known antibiotics are provided. These peptides are related in sequence to amino acid sequences within Cathepsin G. A broad spectrum bactericidal peptide disclosed herein is RPGTLCTVAGWGRVSMRRGT (SEQ ID NO:4); it is active against *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Staphylococcus aureus*. RRENTQQHITARRAIRHPQY (SEQ ID NO:7) and GKSSGVPPEVFTRFVSSFLPWIRTTMR (SEQ ID NO:8) also exhibited potent activiy against *P. aeruginosa* strains, including clinical isolates. The peptides of the present invention will be useful in pharmaceutical compositions useful in the treatment or prophylaxis of infections.

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ANTIMICROBIAL PEPTIDES

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Technical Field

The field of this invention is the area of antimicrobial peptides with activity against a broad range of Gram negative and Gram positive bacteria and fungi. The antimicrobial peptides of this invention are useful for inhibiting microbial growth and in pharmaceutical compositions for treatment or prevention of infections and for the treatment and/or prevention of gingivitis.

Background of the Invention

Microbes which invade the human body are challenged by several defense mechanisms. The nature of the defense mechanisms which any given microbe faces depends on the genetic makeup and the physiologic state of the host as well as the portal of entry of the invading microorganism.

If the mechanical and chemical barriers of the skin or mucous membranes are crossed, immunological factors (e.g.,

nonspecific cellular defenses come into play. Nonspecific cellular defenses in the form of phagocytic white blood cells from local tissues and the bloodstream respond to an invading microbe. Polymorphonuclear leukocytes (PMNs) actively phagocytize particulates such as bacterial or fungal cells. PMNs are the first class of phagocytic cells recruited to the site of infection or inflammation. The PMNs contain azurophilic or primary granules, which contain lysosomal proteases, myeloperoxidase, lysozyme and certain antimicrobial proteins. Secondary granules within these cells contain alkaline phosphatase, lactoferrin and lysozyme. Stores of glycogen within the PMNs provides for energy through glycolysis so that the cell can function in an anaerobic environment.

Adherence of a particle to the surface of a phagocytic cell initiates phagocytosis; the particle enters the cytoplasm in a phagocytic vacuole. This triggers a respiratory burst and the generation of microbicidal metabolites; the primary granule fuses with the phagocytic vacuole to form a digestive vacuole called the phagolysosome. Intracellular killing of the ingested microorganism occurs as a result of oxygen-dependent and oxygen-independent mechanisms. The oxygen-dependent bactericidal halogenating system uses, for example, granule myeloperoxidase, hydrogen peroxide and chloride ion to kill bacteria and viruses via either halogenation of cellular or viral constituents or via reactive oxygen intermediates.

The primary granules contain three major groups of antibacterial proteins. The first group includes catalytically active proteins which are only weakly antibacterial when tested individually in purified form; examples include lysozyme, elastase and collagenase. These enzymes probably participate in the digestion of microorganisms killed by other mechanisms, but elastase, for example, is believed to potentiate killing by the halogenating system. The second category of granule proteins includes

those with catalytic activity and bactericidal activity which is independent of the catalytic activity. An example is the chymotrypsin-like neutral protease of human neutrophils. The third group contains bactericidal members which lack known catalytic activity; included in this class are defensins and cationic antibacterial proteins.

Some cationic antibacterial proteins are of relatively high molecular weight (greater than about 25 kDa) and kill certain Gram negative bacteria, such as Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa, by damaging the cytoplasmic membrane, leading to increased membrane permeability. Human bactericidal/permeability increasing protein (BPI) is a strongly basic protein with molecular weight of about 59 kDa. It is believed that binding of BPI to the outer membrane of susceptible bacterial cells results in exposure of hydrophobic channels through the outer envelope, and as a secondary effect, a selective activation of autolytic enzymes. Gram positive bacteria, certain Gram negative bacteria and fungi are not affected by BPI in vitro.

Low molecular weight cationic proteins (10 kDa to 25 kDa) have been reported to inhibit the multiplication of such Gram positive bacteria as Staphylococcus aureus (Root and Cohen (1981) Rev. Infect. Dis. 3:565-598). In addition, cationic proteins with fungicidal activity have been identified in alveolar macrophages. It is believed that cationic proteins are most efficient in killing phagocytized microorganisms in combination with other microbicidal defense mechanisms (Elsbach and Weiss (1983) supra).

Generally defensins are relatively small polypeptides (3-4 kDa), which are rich in cysteine and arginine. Gabay et al. (1989) Proc. Natl. Acad. Sci. USA 86:5610-5614, used reverse phase HPLC to purify 12 major polypeptides from the azurophil granules of human PMNs; purified proteins were analyzed individually for antimicrobial activity and for N-terminal

amino acid sequence. A 4 kDa defensin (HNP-4) and a 29 kDa polypeptide named azurocidin were purified and shown to possess broad spectrum antimicrobial activity. Defensins as a class have activity against some bacteria, fungi and viruses. They are also reported to have cytotoxic activity against transformed cells. Selsted et al. (1985) J. Clin. Invest. 76:1436-1439, presents a sequence comparison of human and rabbit defensins. The defensins are believed to have molecular conformations stabilized by cystine infrastructure, which are essential for biological activity.

Granzymes are a family of serine proteases in the granules of cytolytic lymphocytes. Proteolytic enzymes are believed to function in cell-mediated cytoxicity; some of the genes have been cloned, and sequence information is available. Within the granzyme family there is at least 38% amino acid sequence identity. Human lymphocyte protease has 73% amino acid sequence identity to mouse granzyme B (Jenne and Tschopp (1988) Immunol. Reviews 103:53-71).

Another class of antimicrobial polypeptides are those known as magainins; at least five proteins can be isolated from the skin of the African clawed frog (Xenopus laevis). The natural proteins are active against a broad range of microorganisms including bacteria, fungi and protozoans (Zasloff (1987) Proc. Natl. Acad. Sci. USA 84:5449-5453). This antimicrobial activity is also present in synthetic peptides and certain truncated analogs of the natural proteins. Derivatives of about 19 to about 23 amino acids have antibacterial activity as measured using Escherichia coli. In the protozoan Paramecium caudatum treated with the magainin peptides, there is disruption of membrane functions. The configurations of the bioactive peptides can be modeled as amphiphilic alpha-helices and are sufficiently long to span a lipid bilayer. (Zasloff et al. (1988) Proc. Natl. Acad. Sci. USA 85:910-913). The sequence of a representative magainin

peptide is GIGKFLHSAKKFKAFVGEIMN (Zasloff et al. (1988) supra) (SEQ ID NO:1).

Cathepsin G (Cat G) is a granule protein with chymotrypsin-like activity; it is also known as chymotrypsin-like cationic protein. Cat G (Odeberg and Olsson (1975) J. Clin. Invest. 56:1118-1124) and three other mutually homologous polypeptides called defensins are active against a broad spectrum of gram positive bacteria, Gram negative bacteria and fungi (Shafer et al. (1986) Infect. Immun. 54:184-188; Shafer et al. (1988) Infect. Immun. 56:51-53; Drazin and Lehrer (1977) Infect. Immun. 17:382-388; Ganz et al. (1986) Semin. Respir. Infect. 1:107-117). Sensitive bacteria include *Capnocytophaga sputigena*, *Escherichia coli*, *Listeria monocytogenes*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa* and *S. aureus*. All of these pathogens, with the notable exceptions of *P. aeruginosa* and *C. sputigena*, are only sensitive to both enzymatically-active and -inactive cathepsin G (Miyasaki and Bodeau (1991) J. Clin. Invest 87:1585-1593; Wasiluk et al. (1991) Infect. Immun. 59:4193-4200 and Table 8). *P. aeruginosa* and *C. sputigena* are only sensitive to enzymatically-active cathepsin G. It is not clear, however, if cathepsin G-killing of these two pathogens requires degradation of bacterial proteins or whether an intact active site is needed to align antibacterial domains of cathepsin G with the bacterial target.

Gabay et al. (1989) supra, has reported antibacterial activities of a number of proteins isolated from human PMNs, including cathepsin G and elastase, and has given the amino terminal sequence of these and other proteins. The N-terminal five amino acids of elastase and Cat G are identical; further sequences have significant relatedness. The amino acid sequence of human Cat G is known, (Salvesen et al. (1987) Biochemistry 26:2289-2293). Sequence analysis of the cDNA revealed significant sequence identity to rat mast cell

proteinase (47%) and to an activated mouse cytotoxic lymphocyte product (56%). Cat G also exhibits significant sequence similarity to chymotrypsin, which is not known to exhibit antimicrobial activity similar to that of Cat G.

As described in WO 91/04414, the Cat G protein was analyzed to determine whether the same portions of the protein were responsible for the enzymatic and antibacterial activity. Purified human Cat G was digested with the proteolytic enzyme clostripain. Peptides resulting from that digestion were purified and individually tested for antibacterial and enzymatic activity. None of the peptides tested exhibited the chymotrypsin-like activity of the intact molecule. However, two Cat G-derived peptides exhibited antibacterial activity using *Staphylococcus aureus* or *Neisseria gonorrhoeae* as the indicator organism. Those peptides were IIGGR (SEQ ID NO:2; amino acids 1-5) and HPQYNQR (SEQ ID NO:3; amino acids 77-83). Antimicrobial activity was maintained with some variation in amino acid sequence, as described in WO 91/04414. Similarly, the oligopeptide corresponding in amino acid sequence to amino acids 1-20 of Cat G exhibited strong bactericidal activity against *Pseudomonas aeruginosa*.

Summary of the Invention

It is an object of this invention to provide oligopeptides with antimicrobial activity. The antimicrobial oligopeptides of the present invention contain from five to about twenty-six amino acids joined in a linear array by peptide bonds, preferably from ten to about twenty-six amino acids.

An object of the present invention is a broad spectrum bactericidal oligopeptide, termed CG 117-136 herein, which has the sequence RPGTLCTVAGWGRVSMRRGT (SEQ ID NO:4). The present invention includes the CG 117-136 oligopeptide in which all

correspondent amino acids are L-amino acids (L-enantiomer) and the CG 117-136 peptide in which all the component amino acids are D-amino acids (D-enantiomer). Further objects are additional bactericidal oligopeptides, particularly effective for P. aeruginosa but not for S. aureus or N. gonorrhoeae, which oligopeptides are termed CG 122-136, CG 127-136, CG 61-80 and CG 198-223, herein. CG 122-136 has the amino acid sequence CTVAGWRGVSMRRGT (SEQ ID NO:5) and CG 127-136 has the amino acid sequence WGRVSMRRGT (SEQ ID NO:6). CG 61-80 has the amino acid sequence RRENTQQHITARRAIRHPQY (SEQ ID NO:7) and CG 196-223 has the sequence GKSSGVPPEVFTRFVSSFLPWIRTTMR (SEQ ID NO:8).

In other embodiments, the antimicrobial oligopeptides comprise the amino acid sequence of CG 1-20, i.e. IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9). CG 1-20 has antimicrobial activity against bacteria including, but not limited to, Pseudomonas aeruginosa and oral pathogens such as Capnocytophaga sputigena, Eikonella corrodens and Actinobacillus actinomycetemcomitans.

D-enantiomers of the antimicrobial oligopeptides of the present invention are particularly preferred.

An object of the present invention is to provide antimicrobial oligopeptides which are useful as bactericides and/or bacteriostats, useful, for example, for killing microorganisms or for inhibiting microbial growth in a variety of solutions and sterile solutions, such as contact lens solutions, herbicidal solutions, hazardous or refuse waste streams, surface disinfectant solutions and oil recovery fluids.

A further object of the invention is to provide therapeutic compositions, suitable for human, veterinary, agricultural or pharmaceutical use, comprising one or more of the antimicrobial oligopeptides of the present invention and a

suitable pharmacological carrier. Such therapeutic compositions can be formulated as understood in the art, e.g., for topical or aerosol application, for controlling and/or preventing infection by Gram positive or Gram negative bacteria or fungi. Preferably, the antimicrobial oligopeptides of the present invention are used in the treatment of infections by Gram negative or Gram positive bacteria. The antimicrobial oligopeptides of the present invention, when used in therapeutic compositions, will not have significant immunogenic activity. In vitro antimicrobial activity of the oligopeptides of the present invention is an accurate predictor of in vivo antimicrobial activity.

Pharmaceutical compositions contain a therapeutically effective amount of an antimicrobial oligopeptide. A therapeutically effective amount of an antimicrobial oligopeptide can be readily determined according to methods known in the art. Pharmaceutical compositions are formulated to contain the therapeutically effective amount of an antimicrobial oligopeptide and a pharmaceutically acceptable carrier appropriate for the route of administration (topical, gingival, intravenous, aerosol, local injection) as known to the art. For agricultural use, the composition comprises a therapeutically effective amount of an antimicrobial oligopeptide and an agriculturally acceptable carrier suitable for the organism (e.g., plant) to be treated. Preferably for use in a pharmaceutical composition, the antimicrobial oligopeptide will have an ED_{50} in vitro less than about 10^{-3} M. The skilled artisan can readily determine a therapeutically effective amount against a target bacterial strain, for example, based on the ED_{50} using the methods disclosed herein and the teachings of the art.

Therapeutic compositions may be administered by topical, dental rinse, aerosol or intravenous application, or by local injection for the control or prevention of infection, by any means known to the art.

IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9) may also be used to kill or control the growth of tumor cells or virus-infected cells. In such applications, these peptides will be particularly useful when coupled to antibodies or other molecules which are specific for the target tumor cell or virus-infected cell so that the peptide acts specifically on the tumor or virus-infected cell.

Brief Description of the Figures

Figure 1 illustrates the complete amino acid sequence for the mature human cathepsin G, as deduced from analysis of its cDNA (See also SEQ ID NO:10). In the chymotrypsin nomenclature, it displays the charge relay profile of His57 Asp102 Ser195 that is typical of serine proteases; the charge relay system amino acids are identified with an asterisk (*). The Ser195 residue (residue 181 in the mature cathepsin G protein) is the target of phosphorylation by DFP, resulting in irreversible inhibition of chymotryptic activity. The residues lining the primary specificity pocket of cathepsin G are marked with #.

Figure 2 presents hydrophobicity analysis for peptide CG 117-136 (SEQ ID NO:4) and the corresponding hydrophobicity-hydrophilicity plot for the amino acid sequence.

Detailed Description of the Invention

As used herein, an oligopeptide is composed of from about five to about twenty-six amino acids linked together by peptide bonds in a linear array. The peptide may be in a linear conformation or it may assume secondary structure. A cyclic peptide derivative can also have antimicrobial activity, and thus is a functional equivalent of the antimicrobial peptides of the present invention. Sequences are conventionally given from the amino terminus to the carboxyl terminus. Component amino acids may be of the D- or

the L-configuration. Unless otherwise noted, the amino acids are L-amino acids. When all component amino acids are of L-configuration, the peptide is said to be an L-enantiomer. When all the amino acids in a peptide are in the D-configuration, that peptide is said to be a D-enantiomer. The peptides of the present invention have antimicrobial activity by themselves or when coupled to another molecule, e.g., polyethylene glycol or a carrier protein such as bovine serum albumin, so long as the peptides are positioned such that they can come into effective contact with the target cell.

Table 1 presents most abbreviations used in this application. Other abbreviations are as commonly used in the art.

TABLE 1
Abbreviations

A	= Ala = Alanine	M	= Met = Methionine
C	= Cys = Cysteine	N	= Asn = Asparagine
D	= Asp = Aspartic Acid	P	= Pro = Proline
E	= Glu = Glutamic Acid	Q	= Gln = Glutamine
F	= Phe = Phenylalanine	R	= Arg = Arginine
G	= Gly = Glycine	S	= Ser = Serine
H	= His = Histidine	T	= Thr = Threonine
I	= Ile = Isoleucine	V	= Val = Valine
K	= Lys = Lysine	W	= Try = Tryptophan
L	= Leu = Leucine	Y	= Tyr = Tyrosine
Boc	= tert-butyloxycarbonyl		
CFU	= colony forming unit		
DFP	= diisopropylfluorophosphate		
HLE	= human leukocyte elastase		
Pam	= (phenylacetamido) methyl		

ED_{50} is the concentration of an antimicrobial agent which kills (or otherwise inhibits growth) 50% of the input indicator microorganisms or cells under particular test conditions.

For convenience, the peptides disclosed herein are named according to the amino acid positions in mature Cat G (Fig. 8).

CG 1-20 represents amino acid residues 1-20 of the mature Cat G sequence and has the sequence IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9).

CG 21-40 corresponds in sequence to amino acids 21-40 of Cat G, QSPAGQSRCGGFLVREDFVL (SEQ ID NO:11).

CG 41-60, corresponding to amino acids 41-60 of Cat G, has the sequence TAAHCWGSNINVTLGAHNIQ (SEQ ID NO:12).

CG 61-80, corresponding to amino acids 61-80 of Cat G, has the sequence RRENTQQHITARRAIRHPQY (SEQ ID NO:7).

CG 77-96, corresponding to amino acids 77-96 of Cat G, has the amino acid sequence HPQYNQRTIQNDIMLLQLSR (SEQ ID NO:13).

CG 97-116, corresponding to amino acids 97-116 of Cat G, has the sequence RVRRNRNVNPVALPRAQEGL (SEQ ID NO:14).

CG 117-136, corresponding to amino acids 117-136 of Cat G, has the sequence RPGTLCTVAGWGRVSMRRGT (SEQ ID NO:4).

CG 137-156, corresponding to amino acids 137-156 of Cat G, has the sequence DTLREVQLRVQRDRQCLRIF (SEQ ID NO:15).

CG 157-176, corresponding to amino acids 157-176 of Cat G, has the sequence GSYDPRRQICVGDRRERKAA (SEQ ID NO:16).

CG 177-197, corresponding to amino acids 177-197 of Cat G, has the sequence FKGDSGGPLLCNNVAHGIVSY (SEQ ID NO:17).

CT 198-223, corresponding to amino acids 198-223 of Cat G, has the sequence GKSSGVPPEVFTRFVSSFLPWIRTTMR (SEQ ID NO:8).

Antimicrobial activity, as used herein, refers to the ability of a peptide of the present invention to kill at least one species selected from the group consisting of Gram positive bacteria, Gram negative bacteria, fungi, and protozoans. It is increasingly preferred that the peptide kill at least 50%, 60%, 70%, 80%, 90% or all cells of at least one species of Gram positive or Gram negative bacteria, fungi, or protozoans. Sensitive Gram positive bacteria can include, but are not limited to, Staphylococcus aureus. Sensitive Gram negative bacteria include, but are not limited to, Escherichia coli, Neisseria gonorrhoeae, and Pseudomonas aeruginosa. Periodontal disease-associated bacteria include Capnocytophaga sputigena, Actinobacillus actinomycetemcomitans and Eikenella corrodens. Capnocytophaga sputigena ATCC 33123 is sensitive to IIGGR (SEQ ID NO:2), IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9) and HPQYNQR (SEQ ID NO:3). A. acetinomycetemcomitans is sensitive to IIGGR and HPQYNQR. E. corrodens is more sensitive to IIGGR than to HPQYNQR. Sensitive fungi can include, but are not limited to, Candida albicans. Antimicrobial activity can also refer to the ability to kill or inhibit the growth of other cells, in particular, those which are tumor cells or virus-infected cells.

The antimicrobial peptides of the present invention are oligopeptides which possess antimicrobial activity, as defined herein. These antimicrobial peptides may contain modifications such as acetylation, provided that the antimicrobial activity is not destroyed. Chemical modifications which do not destroy antimicrobial activity are those which do not substantially decrease the hydrophilicity of the antimicrobial peptide and those which are not bulky

hydrophobic chemical groups, particularly as described in WO 91/04414 for antimicrobial peptides related in sequence to HPQYNQR. Modified peptides with antimicrobial activity are functionally equivalent to the antimicrobial peptides of the present invention. Such modified peptides with antimicrobial activity include, but are not limited to, (1-methyl-H)QYNQR, (3-methyl-H)PQYNQR, (Ac-H)PQYNQR and HPAYNA^{M}K.

Antibacterial pharmaceutical compositions, as defined herein, comprise a pharmaceutically acceptable carrier and one or more antibacterial peptides of the present invention. Such antimicrobial pharmaceutical compositions may be formulated in ways, as understood in the art, for use for topical application, for gingival application (for gingivitis or periodontal disease) or for local or systemic injection. For use in the treatment or prevention of gingivitis, the peptides of the present invention can be incorporated in effective amounts in a dental rinse for application to the buccal area, or they may be incorporated in other suitable compositions for topical application. The antibacterial peptides of the present invention may also be incorporated in effective amounts in chewing gum, lozenges for sucking, toothpowder or toothpaste. The antibacterial peptides of the present invention can comprise from 0.001% to 50% by weight of such compositions. It will be understood that a composition for systemic injection will contain an antimicrobial peptide, e.g., an antibacterial peptide such as CG 117-136, in a therapeutically effective amount or a therapeutically effective amount of an antimicrobial peptide can be conjugated to an antibody, or any other compound as understood in the art, with specificity for the target cell type. The choice of the peptide will be made with consideration of immunogenicity and toxicity to the infected host, effective dose of the peptide, and the sensitivity of the target microbe to the peptide, as well-understood in the art.

Surprisingly, an oligopeptide of the sequence HPQYNQRTIQNDIMLLQLSR (SEQ ID NO:13) did not exhibit significant antimicrobial activity against either S. aureus or P. aeruginosa, although peptides of the sequences HPQYNQ (SEQ ID NO:18) and HPQYNQR (SEQ ID NO:3) were bactericidal.

Cat G-derived peptides were tested for antimicrobial activity against Capnocytophaga sputigena ATCC 33123, which is the same as that now available as C. sputigena ATCC 33612 (American Type Culture Collection, Rockville, Maryland). C. sputigena is representative of oral pathogens associated with periodontal disease and/or gingivitis. IIGGR (SEQ ID NO:2) and CG 77-83 (SEQ ID NO:3) are effective against C. sputigena ATCC 33123 in vitro. When incorporated in pharmaceutical compositions, one or more of the peptides related in sequence to Cat G can be used to ameliorate gingivitis and/or treat periodontal disease. Compositions for oral use include oral rinses, lozenges and formulations for topical application to the gums. The skilled artisan can use the teachings of the present specification and knowledge readily accessible to the art to prepare pharmaceutically useful formulations for oral application, topical or other applications, particularly after animal studies to confirm that these peptides are not toxic to the human or animal host and are effective in vivo.

Because the antimicrobial activity resulting from the IIGGR (SEQ ID NO:2) and HPQYNQR (SEQ ID NO:3) peptides (recovered after clostripain digestion as described in WO 91/04414) was less than 1% of the activity of intact Cat G, an alternate approach was pursued. Eleven peptides spanning the entire 223 amino acid cathepsin G protein were synthesized. These eleven peptides were tested for antibacterial action against N. gonorrhoeae, P. aeruginosa and S. aureus. Of the eleven peptides, only the peptide corresponding to residues 117-136 in the full-length cathepsin G (CG 117-136) (SEQ ID NO:4) displayed antibacterial action against all three pathogens; 500 μg of peptide per ml killed 5-6 logs of P.

aeruginosa and S. aureus. See Table 6 for the peptide sequences and activities, as measured using a crude peptide concentration of 500 μg/ml with an input viable cell concentration of about 10^7 colony forming units/ml (CFU/ml) in 1/100 strength HBSS. CG 117-136 will be useful as an antibacterial agent against a wide range of bacteria, including pathogens.

Table 2

Antibactericidal Activity of Crude Peptides[1]

Peptide Sequence	Log Kill (500 μg/ml)	
	P. aeruginosa ATCC 27853	*N. gonorrhoeae* strain WS1
CG 1-20 (SEQ ID NO:9)	**5.507**	**2.57**
CG 21-40 (SEQ ID NO:11)	-0.034	0.05
CG 41-60 (SEQ ID NO:12)	0.5	-0.09
CG 61-80 (SEQ ID NO:7)	**5.15**	0
CG 77-96 (SEQ ID NO:13)	0.06	0.24
CG 97-116 (SEQ ID NO:14)	**5.93**	-0.21
CG 117-136 (SEQ ID NO:4)	**5.88**	**2.38**
CG 137-156 (SEQ ID NO:15)	0.01	0.43
CG 157-176 (SEQ ID NO:16)	1.02	-0.18
CG 177-197 (SEQ ID NO:17)	-0.79	0.68
CG 198-223 (SEQ ID NO:8)	**5.73**	0.78
Buffer	-0.139	-0.07

[1] Peptides shown in **Bold** were purified by RP-HPLC and again tested vs. *P. aeruginosa*, *N. gonorrhoeae* and *S. aureus* (see Table 3A).

Table 3A provides antimicrobial activities of peptides purified by RP-HPLC, determined as above. Only CG 117-136 (SEQ ID NO:4) exhibits high activity against P. aeruginosa, S. aureus and N. gonorrhoeae. Table 3B discloses the ED_{90} in μg/ml for the antibacterial peptides, as measured with P. aeruginosa ATCC 27853 as above. ED_{90} is the dose required to kill 90% of the input cells in 2 h at 37°C in 1/100 strength HBSS, where the input viable cell concentration is about 10^7 CFU/ml.

Even more effective as an antimicrobial agent than amino acids 1-5 of Cat G is the CG 1-20 oligopeptide (SEQ ID NO:9) but which retains some blocking group(s) from the component derivatized amino acids in chemical peptide synthesis or an artifactual reaction product. The chemical identity of the substituents on that oligopeptide or reaction product have not yet been identified. CG 97-116 (SEQ ID NO:14) also exhibits strong antimicrobial activity as a crude synthetic product, but is not as a purified peptide. The basis for this is also not defined.

Table 3A

Antibacterial Action of RP-HPLC-Purified Peptides

		Log Kill (500 μg/ml)		
		P. aeruginosa	*N. gonorrhoeae*	*S. aureus*
Peptide 8325-4	SEQ ID NO.	ATCC 278533	strain WSI	strain
CG 1-20	9	1.0	0.43	0.3
CG 61-80	7	5.15[1]	ND[2]	0.58
CG 97-116	14	0.22	0.2	-0.1
CG 117-136	4	5.88[1]	4.20	5.34
CG 198-223	8	5.73[1]	ND	0.26
Buffer Control		-0.139	-0.07	-0.25

[1] Also active against four clinical isolates of *P. aeruginosa*
[2] Not determined

Table 3B

Potency of Synthetic Cathepsin G Peptides Against *P. aeruginosa*

Synthetic Peptides	SEQ ID NO.	ED_{90} (μg/ml)
CG 1-20	9	500
CG 61-80	7	75
CG 97-116	14	>500
CG 117-136	4	15
CG 198-223	8	115

It was noted that CG 1-20 (SEQ ID NO:9) and CG 97-116 (SEQ ID NO:14) were highly active as crude peptides, but exhibited much lower activity when purified by RP-HPLC. Without wishing to be bound by any particular hypothesis, the inventors suggest that the high activity in the crude peptide preparation is due to one or more residual blocking or substituent groups or some unidentified side reaction product generated during the hydrogen fluoride cleavage and/or post-synthetic work-up.

Those peptides which exhibited significant activity against P. aeruginosa ATCC 27853, were also tested against four independent clinical isolates. P. aeruginosa ATCC 27853 (American Type Culture Collection, Rockville, Maryland) is the strain used for testing antimicrobial activity against P. aeruginosa unless otherwise noted. It is a standardized strain for antibiotic-susceptibility testing of pseudomonads (see, e.g., Code of Federal Regulations, Title 21, Part 460, 1987). CG 61-80 (SEQ ID NO:7) and CG 198-223 (SEQ ID NO:8) exhibited some variability in effectiveness for killing of clinical strains, but so far as tested, CG 117-136 (SEQ ID NO:4) appeared to be a highly effective bactericidal peptide for P. aeruginosa as well as N. gonorrhoeae and S. aureus (see Tables 3A, 4). Agents effective against P. aeruginosa are needed in the art because multiple antibiotic resistance is quite common among clinical strains, and therefore resultant infections are often difficult to treat.

Table 4
Bactericidal Activity of HPLC-Purified Peptides Against _P._ _aeruginosa_ Clinical Isolates

		Log Reduction in Viability[1]		
_P._ _aeruginosa_	Control	CG 61-80 (SEQ ID NO:7)	CG 117-136 (SEQ ID NO:4)	CG 198-223 (SEQ ID NO:8)
ATCC 27853	-0.14	5.49	5.95	5.35
#385128	-1.1	1.91	6.17	4.71
#36152	-0.89	4.86	5.38	4.28
#27853	-0.58	5.80	5.79	4.80
#A-91-330-0347	-0.41	4.11	6.40	1.17

[1] 3-5 X 10^7 CFU/ml of the designated strains were exposed to 500 μg/ml of HPLC-purified peptides in 1/100 strength BHSS (10 mm sodium phosphate, pH 7.0, 10 mM NaCl.

To further characterize the activity of CG 117-136 (SEQ ID NO:4), a "D-enantiomer," composed only of D-amino acids but in the same sequence, was synthesized and tested for antimicrobial activity. The L- and D-forms of this same amino acid sequence had equivalent bactericidal activity against both _P._ _aeruginosa_ and _N._ _gonorrhoeae_ (Table 5). This result suggests that killing does not require the recognition of a microbial target with a chiral center.

Table 5

The Antibacterial Capacity of CG 117-136 (SEQ ID NO:4) is Independent of Stereochemistry

Peptide	Log Kill[1] *P. aeruginosa* ATCC 27853	*N. gonorrhoeae* strain WS1
L-enantiomer	5.62	4.20
D-enantiomer	5.92	4.20

[1] *P. aeruginosa* (3×10^7 CFU/ml) was exposed to 125 μg/ml of each peptide, while *N. gonorrhoeae* (5×10^6 CFU/ml) was exposed to 500 μg/ml of each peptide.

The D-and L-enantiomers of CG 117-136 (SEQ ID NO:4) were tested for retention of bactericidal activity in the presence of normal mouse serum (NMS). As shown in Table 6, NMS at a concentration of 25% (v/v) in 1/100 HBSS completely inhibited the microbicidal activity of the L-enantiomer. However, the D-enantiomer of CG 117-136 retained significant antimicrobial activity. Without wishing to be bound by any theory, it is suggested that NMS contains endogenous inhibitors or proteases that specifically destroy or inhibit the bactericidal activity of the L-enantiomer of CG 117-136 (SEQ ID NO:4).

Table 6

Bactericidal Activity of L- and D-Enantiomers of CG 117-136 (SEQ ID NO:4) in the Presence of Normal Mouse Serum

Sample	Log Reduction in Viability of _P._ aeruginosa
Bacteria	-0.02
Bacteria + NMS	-0.16
Bacteria + L-enantiomer	≤5.6
Bacteria + L-enantiomer + NMS	-0.19
Bacteria + D-enantiomer	≤5.6
Bacteria + D-enantiomer + NMS	3.80

The L- and D-enantiomeric forms of CG 117-136 (SEQ ID NO:4) were tested for apparent lethality or toxicity to mice. In a pilot experiment, 6 BALB/cAnNCr mice were given an intraperitoneal injection of 500 μg of the L-enantiomeric form of CG 117-136 and 6 BALB/cAnNCr mice were given 500 μg of the D-enantiomeric form of CG 117-136 by intraperiotoneal injection. All 12 test mice remained alive and active at 14 days post injection. Thus, neither the L- nor the D-enantiomer of CG 117-136 has apparent toxicity to mice.

As shown in Fig. 2B and Table 7A, CG 117-136 (SEQ ID NO:4) is predicted to have a hydrophobic domain in the N-terminal portion of the peptide and a cationic, hydrophilic domain in the C-terminal portion. The contribution(s) of these domains to antimicrobial activity was assessed by synthesizing truncated versions of CG 117-136. Both domains were required for full activity. Omission of either C- or N-terminal residues destroyed activity against _S. aureus_, while omission of the five N-terminal residues resulted in only about a 10-fold drop in activity for both _P. aeruginosa_ and _N._

gonorrhoeae. Omission of the ten N-terminal residues caused nearly total loss of activity against P. aeruginosa and a lesser reduction in activity as measured against N. gonorrhoeae (see Table 7A, 7B).

Table 7A

Summary of Domains in CG 117-136 and in Truncated CG 117-136 Variants

Peptide	Hydrophobic Domain	Hydrophilic Domain
CG 117-136 (SEQ ID NO:4)	+	+
CG 117-129 (SEQ ID NO:19)	+	-
CG 122-136 (SEQ ID NO:5)	±	+
CG 127-136 (SEQ ID NO:6)	-	+

Table 7B

Antibacterial Action of CG 117-136 and Truncated Variants of CG 117-136

		Log Kill (500 μg/ml)		
Peptide 8325-4	SEQ ID NO.	*P. aeruginosa* ATCC 27853	*N. gonorrhoeae* strain WSI	*S. aureus* strain
CG 117-136	4	5.8	4.20	5.34
CG 117-129	19	0.86	0.05	0
CG 122-136	5	4.75	4.18	0
CG 127-136	6	0.27	3.20	0

The secondary structure of CG 117-136 (SEQ ID NO:4) has been predicted by computer analysis to exhibit ß-Sheet structure. By contrast, several peptides known to interrupt the Gram negative envelope are α-helical peptides (See, e.g., Vaara, M. (1992) Microbiological Rev. 56:395-411).

The potent antibacterial peptide CG 117-136 (SEQ ID NO:4) displays partial amino acid sequence identities with limited portions of other serine proteases or serine protease-like proteins, termed serpocidins, based on their toxic action against bacteria and eucaryotic cells. The partial sequence identities are less than 50% over the relevant portions, but may be significant. The mechanism of cytotoxicity has not been defined, but it is likely that the serpocidins behave as membrane-disorganizing agents. Electron microscopic analysis of CG 117-136-treated *P. aeruginosa* cells revealed similar effects on cell morphology to those seen after treatment with polycationic agents. Lysis did not result from CG 117-136 treatment.

A comparison of the amino acid sequence of CG 117-136 to partial sequences of other antimicrobial proteins and serine proteases is given in Fig. 3. The dots represent amino acid identity. The skilled artisan can readily identify variants of the exemplified CG 117-136 sequence (SEQ ID NO:4) (or of other antibacterial peptides disclosed herein) by synthesizing defined variants and testing as taught herein.

The sensitivities of the *P. aeruginosa* and *S. aureus* indicator cells to enzymatically-active and inactive Cat G were determined (see Table 8). Surprisingly, peptides CG 61-80, CG 117-136, CG 198-223 (SEQ ID NO:7, SEQ ID NO:4, and SEQ ID NO:8, respectively) from cathepsin G display potent bactericidal action *in vitro* even against a pathogen (*P. aeruginosa*) that is killed only by enzymatically active Cat G.

Table 8

Bactericidal Capacities of Enzymatically-Active and -Inactive Cathepsin G

	Log Reduction in CFU/ml[1]	
	S. aureus	*P. aeruginosa*
Control	-0.18	-0.76
50 μg/ml enzymatically active Cat G	2.82	2.41
50 μg/ml DFP-treated Cat G	2.93	0.125

[1] Approximately 5 X 106 CFU/ml of the test bacteria were incubated with the cathepsin G samples in 1% (w/v) TSB (tryticase soy broth) for 2 h at 37°C. The results are average values from 2 determinations for each strain and preparation of cathepsin G.

Only enzymatically-active cathepsin G kills *P. aeruginosa*, while other pathogens are readily killed by both active and inactive cathepsin G. It is theorized that in order for the antibacterial peptides to properly interact with the pseudomonad cell envelope, the structure of the active site must be in its native state in order to allow accessibility of bactericidal domains in the full-length molecule or promote liberation of bactericidal fragments the full-length molecule by a mechanism such as bacterial protease action or by autoproteolysis, but the inventors do not wish to be bound by this theory. The results disclosed herein support the notion

that bactericidal serine esterases possess broad spectrum antibacterial action due to the presence of internal antibacterial domains and that multiple, distinct domains exist within cathepsin G for the purpose of killing different pathogens.

The following examples are provided for illustrative purposes and are not intended to limit the scope of the invention. Because modification of the examples below will be apparent to those of skill in the art, it is intended that this invention be limited only by the scope of the appended claims. All references cited in this application are hereby incorporated by reference herein.

EXAMPLES

Example 1 Preparation of Synthetic Peptides

Oligopeptides were synthesized using an Applied Biosystems Model 430A peptide synthesizer (0.1 - 0.5 mmol scale) using phenylacetamidomethyl (Pam) or p-methylbenzyhydrylamine copoly(styrene/divinylbenzene) resins (Applied Biosystems, Inc., Foster City, CA) and tert-butyloxycarbonyl (Boc)-protected amino acids (Applied Biosystems, Inc. or Bachem, Inc., Torrance, CA). Boc-N-methyl-Ala, Boc-Arg(tosyl) or Boc-Arg(mesitylenesulfonyl), Boc-Asp(benzyl), Boc-Cys(4-methoxybenzyl), Boc-Glu(benzyl), Boc-His(benzyloxycarbonyl) or Boc-His(2,4-dinitrophenyl), Boc-D-His(4-toluenesulfonyl), Boc-Lys(chlorobenzyloxycarbonyl), Boc-Met, Boc-Ser(benzyl), Boc-Thr(benzyl), Boc-Trp or Boc-Trp(formyl), and Boc-Tyr(2-bromobenzyloxycarbonyl) were used for the incorporation of the respective amino acid residues. Boc-His(methyl) was incorporated in a manual mode on a 0.02 mmol scale using the N,N-dicyclohexylcarbodiimide/1-hydroxybenzotriazole coupling protocol. All amino acids (except glycine) used herein have the L configuration unless otherwise noted.

Peptides were cleaved from the resin and deprotected in liquid HF/p-cresol/dimethyl sulfide (10:1:0.5) at -5°C for 90 min, or in liquid HF/anisole (9:1, v/v) at 0°C for 90 min. The resins were washed with cold diethyl ether, and the peptides were extracted into 1.0 M acetic acid and lyophilized. Peptides were then purified by RP-HPLC on an Aquapore™ RP-300 C18 silica column (1x10 cm, Applied Biosystems, Inc.), or on an MRPH-Gel™ polystyrene column (1 X 10 cm, The Nest Group, Scarborough, MA) using a 0 - 60% linear gradient of acetonitrile in 0.1% TFA. The purity of each synthetic peptide preparation was confirmed by microbore HPLC on Aquapore™ OD-300 columns of C18 silica (1 X 250 mm, Applied Biosystems, Inc.), quantitative amino acid analysis and sequencing, as described above. Peptides were generally stored in the lyophilized form at 4°C prior to use in the antibacterial assays.

It is understood in the art that there are other suitable peptide synthetic devices or that manual peptide synthesis could be carried out to produce the peptides of the present invention. Automated solid phase peptide synthesis is described, e.g., in Stewart et al. (1984) Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, Illinois).

Example 2 Antimicrobial Activity Testing

Neisseria gonorrhoeae strain FA 102 and *Staphylococcus aureus* strain 8325-4 were the test bacteria used in many experiments; these strains have been described previously (Shafer et al. (1986) *supra*; Shafer and Onunka (1989) J. Gen. Microbiol. 135:825-830). *N. gonorrhoeae* were passaged on clear typing agar as nonpiliated, transparent variants. For testing, cultures were grown with shaking at 37°C in GC broth containing glucose, iron and sodium bicarbonate supplements. *S. aureus* was grown at 37°C with shaking in LB broth. At midlogarithmic phase (OD_{550} of 0.35) the cultures were diluted in Hanks Balanced Salt Solution (HBSS) (Gibco Laboratories,

Grand Island, New York) (pH 7.5) to give approximately 10^5 CFU/ml. In other experiments, P. aeruginosa ATCC 27853, a standard antibiotic tester strain, was used.

Peptides were dissolved in HBSS (pH 7.5) and added in various amounts (0 to 100 micrograms) to sterile microtiter wells. After UV sterilization of the wells, 0.1 ml samples of the bacterial were added and the volumes in each well were adjusted with HBSS to 0.2 ml. The bacteria-peptide mixtures were incubated at 37°C for 45-60 min unless otherwise noted. For N. gonorrhoeae, incubation was carried out under an atmosphere of 5% CO_2. In other experiments, as noted, 1/100 strength HBSS was used. For at least some strains, the use of 1/100 HBSS resulted in greater sensitivity to the bactericidal activity of the peptides disclosed herein.

Viability was determined after incubation by plating 10 and 100 microliter samples on LB agar (S. aureus) or GCB agar (N. gonorrhoeae). All assays were done in duplicate or triplicate, and the results given are the means of three independent experiments. The % survival of the test bacteria was calculated as 100 x (# CFU in the presence of peptide)/(# CFU in the absence of peptide); standard error of the mean for each data point was never greater than 5%.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Emory University
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(ii) TITLE OF INVENTION: Antimicrobial Peptides

(iii) NUMBER OF SEQUENCES: 19

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 01-OCT-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/956,848
(B) FILING DATE: 02-OCT-1992

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(C) REFERENCE/DOCKET NUMBER: 3-90C PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 499-8080
(B) TELEFAX: (303) 499-8089

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Gly Ile Gly Lys Phe Leu His Ser Ala Lys Lys Phe Lys Ala Phe
1               5                   10                  15

Val Gly Glu Ile Met Asn
                20
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ile Ile Gly Gly Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
His Pro Gln Tyr Asn Gln Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Arg Pro Gly Thr Leu Cys Thr Val Ala Gly Trp Gly Arg Val Ser
1               5                   10                  15

Met Arg Arg Gly Thr
                20
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Cys Thr Val Ala Gly Trp Arg Gly Val Ser Met Arg Arg Gly Thr
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Trp Gly Arg Val Ser Met Arg Arg Gly Thr
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Arg Arg Glu Asn Thr Gln Gln His Ile Thr Ala Arg Arg Ala Ile
1               5                   10                  15

Arg His Pro Gln Tyr
                20
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Gly Lys Ser Ser Gly Val Pro Pro Glu Val Phe Thr Arg Phe Val
1               5                   10                  15

Ser Ser Phe Leu Pro Trp Ile Arg Thr Thr Met Arg
                20                  25
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ile Ile Gly Gly Arg Glu Ser Arg Pro His Ser Arg Pro Tyr Met
1               5                   10                  15

Ala Tyr Leu Gln Ile
                20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ile Ile Gly Gly Arg Glu Ser Arg Pro His Ser Arg Pro Tyr Met
1               5                   10                  15

Ala Tyr Leu Gln Ile Gln Ser Pro Ala Gly Gln Ser Arg Cys Gly
                20                  25                  30

Gly Phe Leu Val Arg Glu Asp Phe Val Leu Thr Ala Ala His Cys
                35                  40                  45

Trp Gly Ser Asn Ile Asn Val Thr Leu Gly Ala His Asn Ile Asp
                50                  55                  60

Arg Arg Glu Asn Thr Gln Gln His Ile Thr Ala Arg Arg Ala Ile
                65                  70                  75

Arg His Pro Gln Tyr Asn Gln Arg Thr Ile Gln Asn Asp Ile Met
                80                  85                  90

Leu Leu Gln Leu Ser Arg Arg Val Arg Arg Asn Arg Asn Val Asn
                95                  100                 105

Pro Val Ala Leu Pro Arg Ala Gln Glu Gly Leu Arg Pro Gly Thr
                110                 115                 120
```

```
Leu Cys Thr Val Ala Gly Trp Gly Arg Val Ser Met Met Arg Gly
                125                 130                 135

Thr Asp Thr Leu Arg Glu Val Gln Leu Arg Val Gln Arg Asp Arg
                140                 145                 150

Gln Cys Leu Arg Ile Phe Gly Ser Tyr Asp Pro Arg Arg Gln Ile
                155                 160                 165

Cys Val Gly Asp Arg Arg Glu Arg Lys Ala Ala Phe Lys Gly Asp
                170                 175                 180

Ser Gly Gly Pro Leu Leu Cys Asn Asn Val Ala His Gly Ile Val
                185                 190                 195

Ser Tyr Gly Lys Ser Ser Gly Val Pro Pro Glu Val Phe Thr Arg
                200                 205                 210

Val Ser Ser Phe Leu Pro Trp Ile Arg Thr Thr Met Arg
                215                 220
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Gln Ser Pro Ala Gly Gln Ser Arg Cys Gly Gly Phe Leu Val Arg
1               5                   10                  15

Glu Asp Phe Val Leu
                20
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Thr Ala Ala His Cys Trp Gly Ser Asn Ile Asn Val Thr Leu Gly
1               5                   10                  15

Ala His Asn Ile Gln
                20
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
His Pro Gln Tyr Asn Gln Arg Thr Ile Gln Asn Asp Ile Met Leu
1               5                   10                  15

LeuGln Leu Ser Arg
               20
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Arg Val Arg Arg Asn Arg Asn Val Asn Pro Val Ala Leu Pro Arg
1 5 10 15

Ala Gln Glu Gly Leu
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Thr Leu Arg Glu Val Gln Leu Arg Val Gln Arg Asp Arg Gln
1 5 10 15

Cys Leu Arg Ile Phe
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gly Ser Tyr Asp Pro Arg Arg Gln Ile Cys Val Gly Asp Arg Arg
1 5 10 15

Glu Arg Lys Ala Ala
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Phe Lys Gly Asp Ser Gly Gly Pro Leu Leu Cys Asn Asn Val Ala
1               5                   10                  15

His Gly Ile Val Ser Tyr
                20
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
His Pro Gln Tyr Asn Gln
1               5
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg Pro Gly Thr Leu Cys Thr Val Ala Gly Trp Gly Arg
1 5 10

WE CLAIM:

1. An oligopeptide having antimicrobial activity, wherein said peptide comprises a sequence selected from the group consisting of RRENTQQHITARRAIRHPQY (SEQ ID NO:7), GKSSGVPPEVFTRFVSSFLPWIRTTMR (SEQ ID NO:8), CTVAGWGRVSMRGGT (SEQ ID NO:5), WGRSMRGGT (SEQ ID NO:6), RPGTLCTVAGWGRVSMRRGT (SEQ ID NO:4) and D-enantiomers thereof.

2. A method of inhibiting growth of a bacterium, said method comprising the step of exposing a bacterium to at least one antimicrobial oligopeptide of claim 1 in an amount effective for reducing viability of said bacterium at least by 90%, wherein the bacterium is sensitive to the antimicrobial activity of at least one of said peptides.

3. A therapeutic composition suitable for controlling infection by a bacterium, said composition comprising at least one antimicrobial oligopeptide of claim 1 and a pharmacologically acceptable carrier, wherein the bacterium is sensitive to the antimicrobial activity of at least one of said oligopeptides.

4. A method for controlling infection by a bacterium, said method comprising the step of administering a therapeutically effective amount of the composition of claim 3.

5. A method of treating gingivitis and/or periodontitis comprising the step of administering a therapeutically effective amount of a therapeutic composition to an affected patient, wherein said therapeutic composition comprises at least one antimicrobial oligopeptide of claim 1, wherein at least one bacterium involved in the gingivitis and/or periodontitis disease process is sensitive to said oligopeptide.

6. The method of claim 5 wherein said step of administering consists of applying said therapeutic composition to the gums of the affected patient.

7. An oligopeptide having antimicrobial activity, where said oligopeptide comprises an amino acid sequence IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9) or a D-enantiomer thereof.

8. A method of treating gingivitis and/or periodontitis comprising the step of administering a therapeutically effective amount of a therapeutic composition to an affected patient, wherein said therapeutic composition comprises the oligopeptide of claim 7, and wherein at least one bacterium involved in the gingivitis and/or periodontitis disease process is sensitive to IIGGRESRPHSRPYMAYLQI (SEQ ID NO:9).

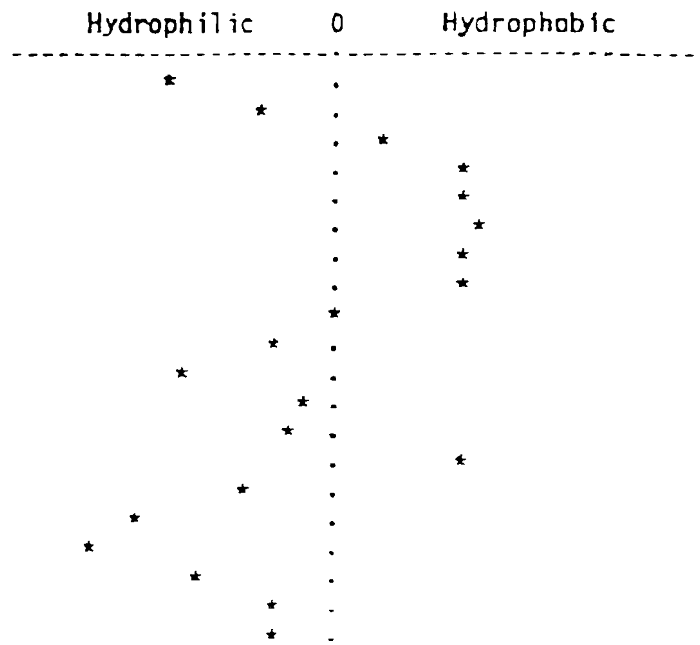
9. A therapeutic composition suitable for controlling infection by a bacterium, said composition comprising the antimicrobial oligopeptide of claim 7 and a pharmacologically suitable carrier wherein the bacterium is sensitive to said oligopeptide.

FIGURE 1

```
                                           *
1
IIGGRESRPHSRPYMAYLQIQSPAGQSRCGGFLVREDFVLTAAHCWGSNINVTLGAHNID

                   80      *                            117
61
RRENTQQHITARRAIRHPQYNQRTIQNDIMLLQLSRRVRRNRNVNPVALPRAQEGLRPGT

                                                     #  ###*
             136
LTVAGWGRVSMMRGTDTLREVQLRVQRDRQCLRIFGSYDPRRQICVGDRRERKAAFKGDS

             ###       #               223
GGPLLCNNVAHGIVSYGKSSGVPPEVFTRVSSFLPWIRTTMR
```

FIGURE 2

Residue	Score	Avg.
117 R	-4.50	-2.167
118 P	-1.60	-0.900
119 G	-0.40	0.900
120 T	-0.70	1.867
121 L	3.80	1.867
122 C	2.50	2.000
123 T	-0.70	1.767
124 V	4.20	1.867
125 A	1.80	0.167
126 G	-0.40	-0.567
127 W	-0.90	-1.933
128 G	-0.40	-0.233
129 R	-4.50	-0.367
130 V	4.20	1.767
131 S	-0.80	-1.133
132 M	1.90	-2.367
133 R	-4.50	-3.133
134 R	-4.50	-1.867
135 G	-0.40	-0.550
136 T	-0.70	-0.700



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09414

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 7/08, 7/10
US CL :514/12, 13, 14; 530/324,326
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 13, 14; 530/324,326

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci., USA, vol. 86, issued July 1989, Gabay et al, "Antibiotic protein of human polymorphonuclear leukocytes", pages 5610-14, see entire document.	1-9
X	Proc. Natl. Acad. Sci. USA, vol. 84, issued April 1987, Sinha et al, "Primary Structure of Human Neutrophil Elastase", pages 2228-32, see entire document.	1-9
X	Biochemistry, vol 26, No.8, issued 1987, "Molecular Cloning of Human Cathepsin G: Structural Similarity to Mast Cell and Cytotoxic T Lymphocyte Proteinases", pages 2289-93, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
10 December 1993

Date of mailing of the international search report
21 DEC 1993

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